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Rapid communication

Monoclonal antibodies against chicken interleukin-6

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Abstract

Monoclonal antibodies (mAb) were produced against a recombinant (r) chicken interleukin-6 (IL-6). Eight mAbs produced were tested for isotype; ability to inhibit recombinant forms of chicken (ch), human (h) and murine (m) IL-6; and recognition of rchIL-6 by Western immunoblotting. The mAb isotypes were represented by IgG_1 (one), IgG_{2a} (six) and IgG_{2b} (one). In a mouse B9 hybridoma cell bioassay with rmIL-6, four mAbs effectively inhibited activity of rmIL-6. Further bioassays with the four mAbs at varying concentrations showed that two of these mAbs (1.20.7 and 1.26.4) were quite effective at inhibiting rmIL-6. Recombinant forms of ch, h and mIL-6 were all tested in a bioassay with the most potent inhibiting mAb (1.26.4), and this mAb was effective in inhibiting all three recombinant IL-6 proteins. Western immunoblotting revealed identification of the original IL-6 immunogen used for mAb production. Based upon inhibition of IL-6 activity in a standard bioassay and IL-6 recognition by Western immunoblotting, mAb 1.26.4 was judged the most useful antibody for future studies and applications.

Keywords: Chicken; Interleukin-6; Monoclonal antibodies

Antibodies against IL-6 have been used to inhibit certain inflammatory responses (Suzuki et al., 1994; Gijbels et al., 1995; Saba et al., 1996; Mazuski et al., 1997; Levi et al., 1998; Marby et al., 2001; Frode et al., 2002; Riedemann et al., 2003) and cancers (Klein et al., 1991; Hudak et al., 1992; Montero-Julian et al., 1995; Smith and Keller, 2001; Trikha et al., 2003; Campo et al., 2005; Wallner et al., 2006) promoted by IL-6 in mammals. Therefore, therapeutic effects of anti-IL-6 molecules have been reported in addition to usefulness of these antibodies in laboratory procedures. A recent report (Nishimichi et al., 2005b) describes a mAb against chIL-6 that recognizes the native form of this chicken cytokine and blocks the active site that binds to IL-6R on a chicken hybridoma cell line. The results presented herein are for mAbs against chIL-6 that recognize the native protein by ELISA and inhibition in a cell proliferation bioassay, and bind to denatured chIL-6 in a Western.

IL-6 cDNA was cloned into pMAL-c2 (New England Biolabs, Beverly, MA) vectors to produce maltose binding protein (MBP) fusion protein containing IL-6. Recombinant chIL-6 was purified using an amylose affinity column (New England Biolabs, Beverly, MA). To develop B-cell hybridomas secreting mouse monoclonal antibodies against chIL-6, BALB/c mice (National Cancer Institute, Fredrick, MD) were immunized biweekly with 50 μg of rchIL-6-MBP emulsified in Freund's adjuvant (Sigma Chemical Co., St. Louis, MO) by intraperitoneal and subcutaneous injections and boosted intravenously with 25 μg of rchIL-6-MBP without adjuvant 3 days prior to fusion. Mice producing high serum Ab titers against chIL-6 were selected by

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ELISA, and their spleen lymphocytes were fused with nonsecreting mouse myeloma SP2/0 cells (ATCC, Rockville, MD).

Hybridomas secreting IL-6 antibodies were cloned by limiting dilution. Ascites were produced in female BALB/c mice following intraperitoneal injection with 0.5 ml of 2,6,14,20-tetramethylpentadecane (Sigma Chemical Co., St. Louis) and a week later with 5×10^6 hybridoma cells. Ascites fluids were purified on fast protein liquid chromatography (FPLC® system; Amersham Pharmacia Biotech, Piscataway, NJ) using HiTrap® Protein G 5-ml column (Amersham Pharmacia Biotech, Piscataway, NJ) according to manufacture's instructions. Isotype of each antibody was determined with isotyping reagents (ISO-2, Sigma Chemical Co., St. Louis).

To screen hybridoma cells, each well of flat-bottom 96-well microtiter plates (Dynex Technologies Inc., Chantilly, VA) was coated with 100 μl of 20 μg/ml of rchIL-6-MBP or MBP alone (negative control) in 0.1 M carbonate buffer, pH 9.6 for 18 h at 4 °C, washed three times with PBS containing 0.05% Tween-20 (Bio-Rad Laboratories, Hercules, CA), pH 7.2 (PBS-T). Each well was blocked with 200 μl of PBS containing 2% BSA for 1 h at room temperature, and washed three times with PBS-T. Hybridoma supernatants (100 μl) were added, the plates incubated for 2 h at room temperature, washed three times. One hundred microlitres of horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (H + L; Sigma Chemical Co., St. Louis) diluted in PBS–0.1% BSA (1:2000) was added and incubated for 1 h at room temperature. Plates were washed three times, 100 μl of 0.01% (w/v) 3,3′,5,5′-tetramethylbenzidine dihydrochloride (Sigma Chemical Co., St. Louis) in 0.05 M phosphate–citrate buffer, pH 5.0 added for 15 min, and reactions stopped with 50 μl of 2 N sulfuric acid.

Interleukin-6 bioactivity was assessed with a B9 bioassay of this cytokine from chicken, and human and murine sources (Roche Diagnostics GmbH, Roche Applied Science, Germany). The mammalian sources were purchased, and rchIL-6 was produced in house. A transformation was performed by addition of 10 ng of plasmid containing the chIL-6 cDNA sequence to one vial of BL21 Star™ (DE3) One Shot® cells. Instructions provided by the Champion™ pET Directional TOPO® Expression kit (Invitrogen Life Technologies, Carlsbad, CA) were followed for expression of rchIL-6.

B9 cells are an IL-6-dependent murine B-cell hybridoma cell line (Aarden et al., 1987). Cells were recovered from flasks and centrifuged for 5 min at $180 \times g$, and the pellet was resuspended in 1–2 ml of IL-6-free culture medium. Following a viability determination by trypan blue exclusion, cells were adjusted to 1×10^5 cells/ml. Anti-chIL-6 antibodies or isotype controls (IgG_1, IgG_{2a}, IgG_{2b}; Sigma Chemical Co., St. Louis) were added in 50 μl volumes in wells within rows of 96-well culture plates leaving empty wells for appropriate controls. Fifty microliters of IL-6 sources at various concentrations were added to appropriate wells. One hundred-microliter volumes of B9 cells (1×10^4) were added to each well along with appropriate control wells containing IL-6-free medium. Plated cells were then placed in a CO_2 (5%) incubator at 37 °C and incubated for 96 h. Four hours prior to termination of the incubation period, 50 μl of MTT (5 mg/ml, tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide, Sigma Chemical Co., St. Louis) were added to each well. At conclusion of incubation, supernatant was removed from each well and 150 μl of DMSO (Dimethylsulfoxide, EMD Chemical Inc., Darmstadt, Germany) was added to lyse cells and dissolve dye crystals. Plates were read on a THERMOmax® (Molecular Devices, Menlo Park, CA) plate reader at 570 and 650 nm and recorded as ΔOD.

For Western blot analysis proteins were mixed with an equal volumes of 0.125 M Tris–HCl, pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol and 0.004% bromophenol blue, heated for 4 min at 94 °C, resolved on 15% SDS-polyacrylamide gels and electroblotted to Immobilon-P membrane (Millipore, Bedford, MA). The membrane was blocked with PBS containing 1% nonfat dry milk for 16 h at 4 °C, incubated with undiluted hybridoma culture supernatants, washed with PBS-T and bound antibody reacted with HRP-conjugated goat anti-mouse IgG antibody in PBS–BSA for 30 min at room temperature. The membrane was washed five times with PBS-T and five times with distilled water and developed using 3,3′-diaminobenzidine substrate (Sigma Chemical Co., St. Louis).

After initial screening of monoclonal antibodies for their reactivity against chIL-6, eight mAbs which showed high binding activities against rchIL-6 in the ELISA were selected for further characterization. Antibody 1.12.4 was determined to be IgG_1, antibody 1.26.4 was isotyped as IgG_{2b}, and the remaining six antibodies (1.15.4, 1.20.7, 1.22.2, 1.24.5, 1.28.7, and 1.31.5) were found to be IgG_{2a}.

A B9 bioassay with rmIL-6 was conducted with inclusion of the eight mAbs, isotype controls and a negative antibody/Ig control (Fig. 1). The negative antibody/Ig control and three isotype controls had similar stimulation indices (SI). Antibodies 1.24.5 and 1.28.7 did not inhibit activity of IL-6 in the B9 bioassay,

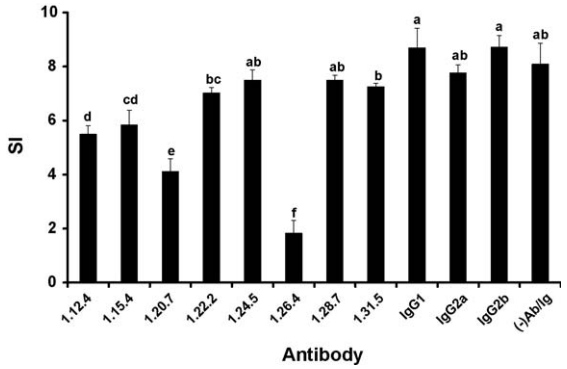

Fig. 1. The effects of different monoclonal antibodies produced against recombinant chicken interleukin-6 (IL-6) and isotype controls (10 μg/ml) on activity of murine IL-6 (0.05 ng/ml) stimulation (SI, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide OD of IL-6 stimulated cells/OD of non-stimulated cells) of IL-6-dependent murine B9 hybridoma cells in culture. B9 cells were co-cultured with antibodies/isotypes and murine IL-6 for 96 h at 37 °C (5% CO_2). Bars with different letters are significantly different at $P \leq 0.05$.

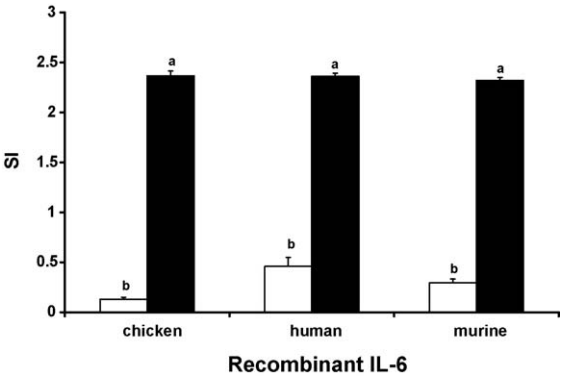

Fig. 2. The effects of monoclonal antibody 1.26.4 (open bars) and isotype control IgG_{2b} (closed bars) at 3.75 μg/ml on the activity of chicken, human and murine IL-6 (0.025 ng/ml) stimulation (SI, OD of IL-6 stimulated cells/OD of non-stimulated cells) of IL-6-dependent murine B9 hybridoma cells in culture. B9 cells were co-cultured with antibody/isotype and IL-6 sources for 96 h at 37 °C (5% CO_2). Bars with different letters within IL-6 source are significantly different at $P \leq 0.05$.

and antibodies 1.22.2 and 1.31.5 did not significantly lower activity of IL-6 on B9 cells compared to 1.24.5 and 1.28.7 mAbs. Significantly lower responses of B9 cells to IL-6, compared to controls, were seen with antibodies 1.12.4 and 1.15.4. The most significant inhibition/neutralization of IL-6 in the bioassay system was observed with antibodies 1.20.7 and 1.26.4.

With identification of four antibodies (1.12.4, 1.15.4, 1.20.7 and 1.26.4) having inhibitory activity on rmIL-6, an assay was run with varying concentrations of these four antibodies in order to determine an effective concentration for neutralizing IL-6 (Table 1). Within each antibody tested, it is apparent that both 1.20.7 and 1.26.4 were effectively inhibiting IL-6 at higher concentrations of each of these antibodies. Overall, antibody 1.26.4 was best at neutralizing action of rmIL-6 in the bioassay.

Using antibody 1.26.4 as the best neutralizing reagent, a B9 bioassay was conducted to neutralize bioactivity of recombinant ch, h and mIL-6 sources. Antibody 1.26.4 was very effective in neutralizing all three recombinant IL-6 sources (Fig. 2). It is interesting to find that mAbs produced against chIL-6 effectively inhibited h and mIL-6. Schneider et al. (2001) had shown that chIL-6 is 52 and 44% similar to human and rat, respectively, with 35 and 25% identity, respectively. So, neutralizing antibodies in this study are not only inhibiting activity of different IL-6 sources, but these antibodies are binding to relevantly identical site(s) on ch, h and mIL-6 molecules.

Antibody 1.26.4 provided positive identification of rchIL-6 (Fig. 3). Lane 1 shows recognition of MBP/IL-6 fusion protein by antibody 1.26.4, whereas lane 2 was

Table 1
The effects of different concentrations (μg/ml) of monoclonal antibodies produced against recombinant chicken IL-6 to neutralized recombinant murine IL-6 (0.025 ng/ml) in a 96-h murine B9 hybridoma cell bioassay

Antibody/isotype	Antibody/isotype concentration (μg/ml)				
	0.9375	1.875	3.75	7.5	15
1.12.4	$6.20^{a} \pm 1.53$	$5.72^{a} \pm 1.31$	$5.49^{a} \pm 1.01$	$4.58^{a} \pm 0.83$	$2.75^{a} \pm 0.38$
1.15.4	$6.25^{a} \pm 1.60$	$5.97^{a} \pm 1.18$	$5.48^{a} \pm 1.05$	$5.23^{a} \pm 0.85$	$5.10^{a} \pm 0.53$
1.20.7	$7.66^{a} \pm 1.54$	$7.17^{a} \pm 1.54$	$5.88^{a} \pm 0.99$	$4.78^{ab} \pm 0.61$	$2.02^{b} \pm 0.25$
1.26.4	$4.01^{a} \pm 0.60$	$3.55^{a} \pm 0.75$	$2.43^{ab} \pm 0.44$	$1.12^{b} \pm 0.08$	$0.66^{b} \pm 0.08$
IgG1	$6.83^{a} \pm 1.91$	$6.46^{a} \pm 1.43$	$6.39^{a} \pm 1.65$	$6.07^{a} \pm 1.30$	$4.83^{a} \pm 1.05$
IgG2a	$7.40^{a} \pm 1.54$	$6.39^{a} \pm 1.33$	$6.42^{a} \pm 1.06$	$6.84^{a} \pm 1.26$	$5.93^{a} \pm 0.98$
IgG2b	$6.71^{a} \pm 1.41$	$6.91^{a} \pm 1.32$	$6.55^{a} \pm 1.26$	$6.88^{a} \pm 0.08$	$6.08^{a} \pm 0.91$

Values (means ± S.E.M.) are reported as stimulation indices of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide optical density (OD) of IL-6 stimulated B9 cells divided by OD of unstimulated B9 cells. Murine IL-6 Control, SI = 6.72. Means with different superscripts (a,b) are different ($P \leq 0.05$) within antibody across concentrations.

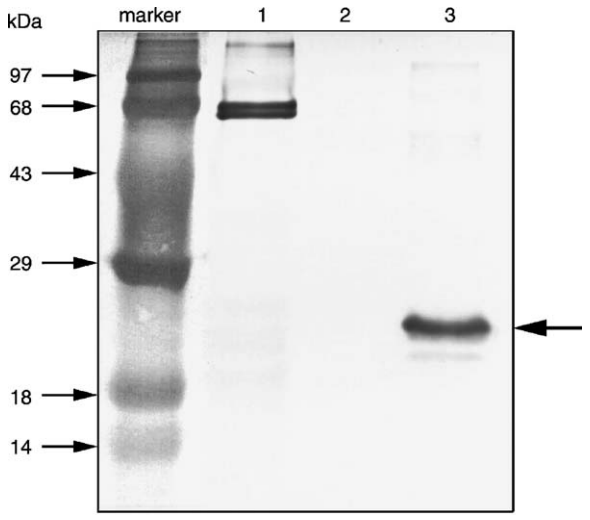

Fig. 3. Western immunoblot analysis of anti-IL-6 monoclonal antibody 1.26.4. Lane 1, intact recombinant chicken IL-6-MBP fusion protein stained with mAb 1.26.4; lane 2, recombinant chicken IL-6 protein stained with anti-IFN-γ mAb; lane 3, recombinant chicken IL-6 after Factor Xa digestion (Song et al., 1997) stained with mAb 1.26.4.

stained with mAb detecting chIFN-γ (Yun et al., 2000) as negative control. Lane 3 shows mAb 1.26.4 recognition of rchIL-6, which was cleaved for MBP (Song et al., 1997) to give the predicted MW for rchIL-6. The mAb 1.26.4 recognized a denatured form of rchIL-6 in this Western.

The screening ELISA showed that the native form of recombinant protein was recognized by all eight of the antibodies originally identified for neutralization assays and Western immunoblots. Therefore, utility of these antibodies is such that multiple assay techniques could be employed with them to evaluate naturally occurring chIL-6 in various specimens (e.g., blood and tissues). As of yet, detection of natural chIL-6 by 1.26.4 has not been determined. Often, a mAb will recognize native protein, but not have neutralizing ability nor be able to bind to a denatured form of the protein. A recent article (Nishimichi et al., 2005b) reports a mAb against chIL-6 that recognizes native protein and inhibits the cytokine's ability to bind to IL-6R on a chicken hybridoma cell line (HUC2-13; Nishimichi et al., 2005a). The most useful reagents allow researchers to identify protein in various different procedures using both native and denatured proteins of natural and recombinant forms.

The effectiveness of mAbs in vivo is another aspect of antibody usefulness as an immunotherapy. Although it is not likely that these antibodies against chIL-6 will be practical as anti-inflammatory reagents on a large-scale industry basis in chickens, these antibodies will be useful for diagnostic tests and quick assays (i.e., colormetric tests) to screen for inflammatory responses in poultry. There are various situations in poultry health practice where vaccines or nutrient feed contents can lead to induction of undesired inflammatory reactions. Current literature is rapidly accumulating on detection of cytokines like IL-6 in cells and tissues of chickens by quantitative PCR techniques (Kaiser et al., 2000, 2002; Okamura et al., 2003; Sijben et al., 2003; Lam, 2004; Jarosinski et al., 2005; Khatri and Palmquist, 2005; Kogut et al., 2005a,b; Rebel et al., 2005; Eldaghayes et al., 2006; Hangalapura et al., 2006). This method of cytokine detection and quantitation is very sensitive and provides very meaningful data on expression of cytokines under various treatments and conditions. However, not everyone has access to this type of technology, and use of a more generalized reagent like a mAb would provide an approach to detecting IL-6 in specimens while the quantitative PCR approach becomes more affordable and applicable to field-like conditions. No doubt with intense interest in nanotechnology, a convenient methodology will be available in the future.

Presently, one or more of the mAbs described in this report can be used to detect and screen for IL-6 in chickens. On a broader scale, there is cross-reactivity with h and mIL-6, which allows for the use of one or two of these antibodies for evaluations of h and mIL-6 as well.

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References

Aarden, L.A., De Groot, E.R., Schaap, O.L., Lansdorp, P.M., 1987. Production of hybridoma growth factor by human monocytes. Eur. J. Immunol. 17, 1411–1416.

Campo, S., Serlupi-Crescenzi, O., Arseni, B., Rossi, S., Saggio, I., Salone, B., Cherubini, G., Carminati, P., De Santis, R., 2005. Comparative activity of Stant7 and anti-IL-6, IL-6R monoclonal antibodies in a murine model of B-cell lymphoma. Cytokine 7, 368–374.

Eldaghayes, I., Rothwell, L., Williams, A., Withers, D., Balu, S., Davison, F., Kaiser, P., 2006. Infectious bursal disease virus: strains that differ in virulence differentially modulate the innate immune response to infection in the chicken bursa. Viral Immunol. 19, 83–91.

Frode, T.S., Souza, G.E., Calixto, J.B., 2002. The effects of IL-6 and IL-10 and their specific antibodies in the acute inflammatory

response induced by carrageenan in the mouse model of pleurisy. Cytokine 17, 149–156.

Gijbels, K., Brocke, S., Abrams, J.S., Steinman, L., 1995. Administration of neutralizing antibodies to interleukin-6 (IL-6) reduces experimental autoimmune encephalomyelitis and is associated with elevated levels of IL-6 bioactivity in central nervous system and circulation. Mol. Med. 1, 795–805.

Hangalapura, B., Kaiser, M., van der Poel, J., Parmentier, H., Lamont, S., 2006. Cold stress equally enhances in vivo pro-inflammatory cytokine gene expression in chicken lines divergently selected for antibody responses. Develop. Comp. Immunol. 30, 503–511.

Hudak, S., Thompson-Snipes, L., Rocco, C., Jackson, J., Pearce, M., Rennick, D., 1992. Anti-IL-6 antibodies suppress myeloid cell production and the generation of CFU-c in long-term bone marrow cultures. Exp. Hematol. 20, 412–417.

Jarosinski, K.W., Njaa, B.L., O'Connell, P.H., Schat, K.A., 2005. Pro-inflammatory responses in chicken spleen and brain tissues after infection with very virulent plus Marek's disease virus. Viral Immunol. 18, 148–161.

Kaiser, P., Rothwell, L., Galyov, E.E., Barrow, P.A., Burnside, J., Wigley, P., 2000. Differential cytokine expression in avian cells in response to invasion by *Salmonella typhimurium*, *Salmonella enteriditis* and *Salmonella gallinarum*. Microbiologica 146, 3217–3226.

Kaiser, P., Underwood, G., Davison, F., 2002. Differential cytokine responses following Marek's disease virus infection of chickens differing in resistance to Marek's disease. J. Virol. 77, 762–768.

Khatri, M.J., Palmquist, M., Cha, R.M., Sharma, J.M., 2005. Infection and activation of bursal macrophages by virulent infectious bursal disease virus. Virus Res. 113, 44–50.

Klein, B., Wijdenes, J., Zhang, X.G., Jourdan, M., Boiron, J.M., Brochier, J., Liautard, J., Merlin, M., Clement, C., Morel-Fournier, B., Lu, X.Y., Mannoni, P., Sany, J., Bataille, R., 1991. Murine anti-interleukin-6 monoclonal antibody therapy for a patient with plasma cell leukemia. Blood 78, 1198–1204.

Kogut, M.H., He, H., Kaiser, P., 2005a. Lipopolysaccharide binding protein/CD14/TLR4-dependent recognition of salmonella LPS induces the functional activation of chicken heterophils and up-regulation of pro-inflammatory cytokines and chemokine gene expression in these cells. Anim. Biotechnol. 16, 165–181.

Kogut, M.H., Iqbal, M., He, H., Philbin, V., Kaiser, P., Smith, A., 2005b. Expression and function of Toll-like receptors in chicken heterophils. Develop. Comp. Immunol. 29, 791–807.

Lam, L.M., 2004. *Mycoplasma gallisepticum*-induced alterations in cytokine genes in chicken cells and embryos. Avian Dis. 48, 215–219.

Levi, M., van Der Poll, T., ten Cate, H., Kuipers, B., Biemond, B.J., Jansen, H.M., ten Cate, J.W., 1998. Differential effects of anti-cytokine treatment on bronchoalveolar hemostasis in endotoxemic chimpanzees. Am. J. Respir. Crit. Care Med. 158, 92–98.

Marby, D., Lockhart, G.R., Raymond, R., Linakis, J.G., 2001. Anti-interleukin-6 antibodies attenuate inflammation in a rat meningitis model. Acad. Emerg. Med. 8, 946–949.

Mazuski, J.E., Tolman, K., Shapiro, M.J., 1997. Effects of cytokine antagonists on the hepatic acute-phase response. J. Surg. Res. 68, 161–169.

Montero-Julian, F.A., Klein, B., Gautherot, E., Brailly, H., 1995. Pharmacokinetic study of anti-interleukin-6 (IL-6) therapy with monoclonal antibodies: enhancement of IL-6 clearance by cocktails of anti-IL-6 antibodies. Blood 85, 917–924.

Nishimichi, N., Aosasa, M., Kawashima, T., Horiuchi, H., Furusawa, S., Matsuda, H., 2005a. Biological activity of recombinant chicken interleukin-6 in chicken hybridoma cells. Vet. Immunol. Immunopathol. 106, 97–105.

Nishimichi, N., Aosasa, M., Kawashima, T., Horiuchi, H., Furusawa, S., Matsuda, H., 2005b. Generation of a mouse monoclonal antibody against chicken interleukin-6. Hybridoma 24, 115–117.

Okamura, M., Lillehoj, H.S., Raybourne, R.B., Babu, U., Heckert, R., 2003. Antigen-specific lymphocyte proliferation and interleukin production in chickens immunized with killed *Salmonella enteriditis* vaccine or experimental subunit vaccines. Avian Dis. 47, 1331–1338.

Rebel, J.M.J., Balk, F.R.M., Boersma, W.J.A., 2005. Cytokine responses in broiler lines that differ in susceptibility to malabsorption syndrome. Br. Poult. Sci. 46, 679–686.

Riedemann, N.C., Neff, T.A., Guo, R.F., Bernacki, K.D., Laudes, I.J., Sarma, J.V., Lambris, J.D., Ward, P.A., 2003. Protective effects of IL-6 blockade in sepsis are linked to reduced C5a receptor expression. J. Immunol. 170, 503–507.

Saba, A.A., Kaidi, A.A., Godziachvili, V., Dombi, G.W., Dawe, E.J., Libcke, J.H., Silva, Y.J., 1996. Effects of interleukin-6 and its neutralizing antibodies on peritoneal adhesion formation and wound healing. Am. Surg. 62, 569–572.

Schneider, K., Klaas, R., Kaspers, B., Staeheli, P., 2001. Chicken interleukin-6: cDNA structure and biological properties. Eur. J. Biochem. 268, 4200–4206.

Sijben, J.W.C., Klasing, K.C., Schrama, J.W., Parmentier, H.K., van der Poel, J.J., Savelkoul, H.F.J., Kaiser, P., 2003. Early in vivo cytokine genes expression in chickens after challenge with Salmonella typhimurium lipopolysaccharide and modulation by dietary *n*-3 polyunsaturated fatty acids. Develop. Comp. Immunol. 27, 611–619.

Smith, P.C., Keller, E.T., 2001. Anti-interleukin-6 monoclonal antibody induces regression of human prostate cancer xenografts in nude mice. Prostate 48, 47–53.

Song, K.D., Lillehoj, H.S., Choi, K.D., Zarlenga, D., Han, J.Y., 1997. Expression and functional characterization of recombinant chicken interferon-gamma. Vet. Immunol. Immunopathol. 58, 321–333.

Suzuki, Y., Yang, Q., Conley, F.K., Abrams, J.S., Remington, J.S., 1994. Antibody against interleukin-6 reduces inflammation and numbers of cysts in brains of mice with toxoplasmic encephalitis. Infect. Immun. 62, 2773–2778.

Trikha, M., Corringham, R., Klein, B., Rossi, J.F., 2003. Targeted anti-interleukin-6 monoclonal antibody threrapy for cancer: a review of the rationale and clinical evidence. Clin. Cancer Res. 15, 4653–4665.

Wallner, L., Dai, J., Escara-Wilke, J., Zhang, J., Yao, Z., Lu, Y., Trikha, M., Nemeth, J.A., Zaki, M.H., Keller, E.T., 2006. Inhibition of interleukin-6 with CNTO328, an anti-interleukin-6 monoclonal antibody, inhibits conversion of androgen-dependent prostate cancer to an androgen-independent phenotype in orchiectomized mice. Cancer Res. 66, 3087–3095.

Yun, C.H., Lillehoj, H.S., Choi, K.D., 2000. Chicken IFN-γ monoclonal antibodies and their application in enzyme-linked immunosorbent assay. Vet. Immunol. Immunopathol. 73, 297–308.